Research paper

The aminopyridine-3,5-dicarbonitrile core for the design of new non-nucleoside-like agonists of the human adenosine A$_{2B}$ receptor

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ABSTRACT

A new series of amino-3,5-dicyanopyridines (**3**–**28**) as analogues of the adenosine hA$_{2B}$ receptor agonist BAY60-6583 (compound **1**) was synthesized. All the compounds that interact with the hA$_{2B}$ adenosine receptor display EC$_{50}$ values in the range 9–350 nM behaving as partial agonists, with the only exception being the 2-{[4-(4-acetamidophenyl)-6-amino-3,5-dicyanopyridin-2-yl]thio}acetamide (**8**) which shows a full agonist profile. Moreover, the 2-[(1H-imidazol-2-yl)methylthio)]-6-amino-4-(4-cyclopropylmethoxy-phenyl)pyridine-3,5-dicarbonitrile (**15**) turns out to be 3-fold more active than **1** although less selective. This result can be considered a real breakthrough due to the currently limited number of non-adenosine hA$_{2B}$ AR agonists reported in literature. To simulate the binding mode of nucleoside and non-nucleoside agonists at the hA$_{2B}$ AR, molecular docking studies were performed at homology models of this AR subtype developed by using two crystal structures of agonist-bound A$_{2A}$ AR as templates. These investigations allowed us to represent a hypothetical binding mode of hA$_{2B}$ receptor agonists belonging to the amino-3,5-dicyanopyridine series and to rationalize the observed SAR.

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1. Introduction

Adenosine (Ado) is an endogenous purine nucleoside that normally increases under pathological or stressful situations producing its effects through activation of G protein–coupled adenosine receptors (ARs). These latter, classified as A$_1$, A$_{2A}$, A$_{2B}$, and A$_3$, are typically coupled to adenylate cyclase but other second messenger systems have also been described [1,2]. Over the years, many ligands, agonists and antagonists, have been identified for the A$_1$, A$_{2A}$, and A$_3$ ARs that, in turn, have been extensively characterized [3]. In contrast, the A$_{2B}$ AR subtype is the least known. In fact, while a large number of selective A$_{2B}$ AR antagonists belonging to

different chemical classes has been developed [4–10], only a few A$_{2B}$ AR agonists are known so far [11]. As antagonists are characterized by a large structural variability, the agonist profile has been long associated to an Ado-like structure. Starting from the 5′-(N-ethylcarboxamido)adenosine (NECA), the first Ado-derived nucleosidic human (h) A$_{2B}$ AR agonist, a slightly more potent hA$_{2B}$ agonist than NECA was identified [12]. Fortunately, progress has been made. In fact, the non-Ado-like 2-{[6-Amino-3,5-dicyano-4-(4-(cyclopropylmethoxy)phenyl)pyridin-2-yl]thio}acetamide (BAY60-6583, compound **1**), a 2-aminopyridine-3,5-dicarbonitrile derivative (Chart 1) discovered by Bayer Healthcare [13,14], is the only available potent and selective hA$_{2B}$ AR agonist reported so far. Its identification has invalidated the conviction that the sugar moiety is essential for agonism at ARs, such that non-nucleoside ligands must therefore behave as antagonists. Thus, compound **1** has been used extensively as a research tool to clarify the pharmacological roles of A$_{2B}$ AR [15–28] sometimes leading to contradictory results [29]. Thus, it could be a very important goal to obtain other potent and selective hA$_{2B}$ AR agonists especially considering the difficulties that have emerged in understanding of the
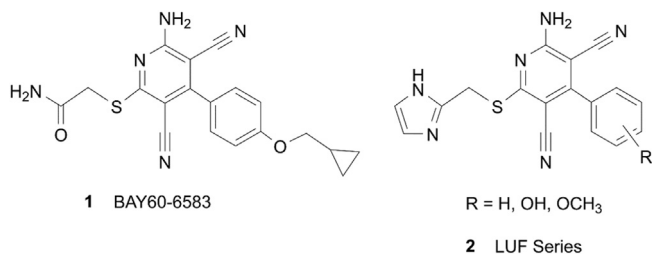
Chart 1. Lead structures for the development of currently reported amino-3,5-dicyanopyridine-based AR ligands.

Chart 2. Modification performed at R^1 and R^2 positions of the 2-amino-4-aryl-6-sulfanyl-3,5-dicyanopyridine scaffold.

pharmacological properties of A_{2B} AR agonists and the necessity to explain some controversies concerning the A_{2B}AR [29]. In particular, the amino-3,5-dicyanopyridine series, to which compound **1** belongs, has been demonstrated to include partial agonists with a variable maximum agonist effect at the hA_{2B} AR subtype [30]. More recently, two different papers reported the partial agonist profile of **1** [27,31] and also its potential A_{2B} AR biased agonism was hypothesized [29,32].

In this scenario, our research group focused attention on the aminopyridine-3,5-dicarbonitrile series to broaden the scarcely known structure-activity relationships (SARs) of this chemical class. In fact, most of the non-Ado-like AR ligands belonging to this series are included in patent documents [12,13,21] while few data are reported in the open literature [30,33,34]. These are, however, sufficient to underline the versatility of the amino-3,5-dicyanopyridine scaffold for producing AR ligands with not only a wide range of affinities but, interestingly, with different degrees of efficacy, ranging from full to partial agonist or neutral antagonist at the different ARs. In particular, certain 2-amino-4-aryl-6-(1H-imidazol-2-yl-methylsulfanyl)-pyridine-3,5-dicarbonitriles belonging to the LUF series [30] (**2**, Chart 1) displayed nanomolar affinity for all the ARs, including the A_{2B} AR subtype on which they showed, in general, also considerable efficacy. Moreover, this class of compounds seems to be more versatile for pharmacological studies showing less species differences than the Ado-like AR agonists [3]. It is worth noting that in addition to compound **1**, that reached preclinical-phase investigation for treating angina pectoris, also other amino-3,5-dicyanopyridine derivatives discovered by Bayer Healthcare have attracted attention for their potential in heart diseases [32,34].

Thus, taking compound **1** as lead, modifications on the amino-3,5-dicyanopyridine core were performed at both R^1 and R^2 positions (compounds **3–28**, Chart 2).

2. Results and discussion

2.1. Chemistry

The synthetic pathways which yielded compounds **1**, **3–28**, **52** and the relative intermediates are illustrated in Schemes 1–3. The amino-3,5-dicyanopyridine derivatives **1**, **3–28** [13,21] (Scheme 1) were obtained starting from aldehydes **29–35**, all commercially available with the exception of the 4-(cyclobuthylmethoxy)benzaldehyde **29** [35] which was obtained by reacting 4-hydroxybenzaldehyde with (bromomethyl)cyclobutane in refluxing acetone and in the presence of potassium carbonate. By one-pot cyclization of the suitable aldehyde **29–33**, **35** with malononitrile and thiophenol, the sulfanylphenyl intermediates **37–41**, **43** were obtained. Different cyclization alkaline adjuvants able to work in a phase-transfer system were used, the best being DBU [36]. Moderate to good yields were obtained. Differently, the *para*-
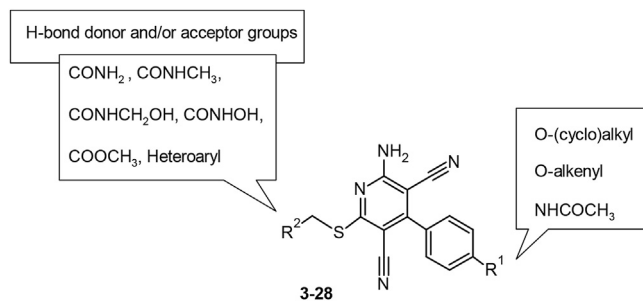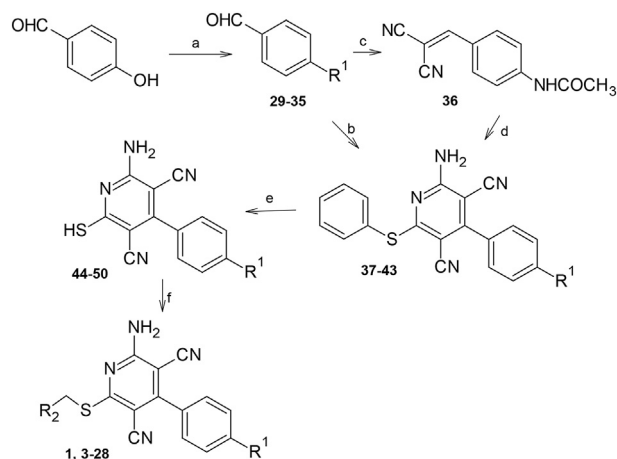
acetamido-benzaldehyde **34** was reacted with malononitrile in a straightforward Knoevenagel condensation in the presence of a few drops of piperidine as catalyst to give the intermediate **36** [37]. The latter was reacted with malononitrile in a cyclization reaction involving thiophenol and Et_3N to afford **42** [37].

To obtain the free thiols (compounds **44–50** [13,37,38]), the corresponding 6-phenylsulfanyl derivatives **37–43** [37] were treated with sodium sulfide in DMF at 80 °C followed by 1 M HCl. The final compounds **3–28** were obtained by reaction of the 6-thiol-derivatives **44–50** with the suitable halides in the presence of sodium hydrogencarbonate. These latter were all commercially available with the exception of the 2-chloro-N-hydroxyacetamide **51** [39] which was synthesized from ethyl 2-chloroacetate with 50% aqueous solution of hydroxylamine as reported in Scheme 2.

Moreover, the hA_{2B} AR agonist **1** [13,21] was cyclized in absolute ethanolic potassium hydroxide to yield the bicyclic compound **52** (Scheme 3). The forced alkaline conditions produced the condensation of the 3-cyano substituent with the active methylene group

R^1	compound	R^1	compound
(cyclobutylmethoxy)	**3, 29, 37, 44**	(allyloxy)	**7, 33, 41, 48**
(isobutoxy)	**4, 30, 38, 45**	NHCOCH₃	**8, 34, 36, 42, 49**
(propoxy)	**5, 25-27, 31, 39, 46**	(cyclopropylmethoxy)	**1, 9-24, 35, 43, 50**
(butoxy)	**6, 28, 32, 40, 47**		

Scheme 1. Reagents and conditions. a) To yield compound **29**: $BrCH_2C_4H_7$, acetone, anhydrous K_2CO_3, reflux (67%); compounds **30–35** are commercially available; b) malononitrile, thiophenol, DBU, 10% aqueous EtOH, 55 °C (18–37%); c) malononitrile, piperidine, EtOH, 80 °C (63%); d) malononitrile, thiophenol, Et_3N, EtOH, reflux (44%); e) Na_2S, anhydrous DMF, 80 °C; 1 M HCl, rt (72–86%); f) R_2CH_2X (X = Cl, Br), $NaHCO_3$, anhydrous DMF, rt (19–80%).